

cedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0172] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0173] Various compounds of the present invention have been found to exhibit antibacterial activity and prevent the growth and spore germination of bacteria such as *Bacillus*, *Burkholderia*, *Enterobacter*, *Escherichia*, *Helicobacter*, *Klebsiella*, *Mycobacterium*, *Neisseria*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Yersinia* and the like, including drug resistance strains. In preferred embodiments, the bacteria is *B. anthracis* (including Ames strain and ciprofloxacin resistant Ames strain) *B. anth1024*, *B. brevis*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis*, *B. vollum*, and spores thereof; *B. cepacia*, *B. mallei*, *M. pseudomallei*, and *B. thailandensis*; *E. coli*, *E. faecalis*, *E. faecium*, and vancomycin resistant strains thereof; *K. pneumoniae*; *P. aeruginosa*, preferably PAO1; *S. aureus* and methicillin resistant *S. aureus*; *Y. pestis*; or a combination thereof. Various compounds of the present invention, including NSC 240898, NSC 266474, NSC 266476, NSC 290107, NSC 290108, NSC 290109, NSC 294200, NSC 294201, NSC 294203, NSC 294204, NSC 294206, NSC 300511, NSC 308571, NSC 308572, NSC 308574, NSC 317880, NSC 317881, NSC 317884, NSC 317885, 317886, NSC 317887, NSC 341907, NSC 341909, and NSC 341911 are also found to inhibit the protease activity of anthrax lethal factor.

[0174] Thus, not only is the present invention directed to methods of inhibiting toxin activity, such as botulinum neurotoxin serotype A light chain metalloprotease activity or the protease activity of anthrax lethal factor, but it is also directed to methods of treating a subject suffering from a bacterial infection.

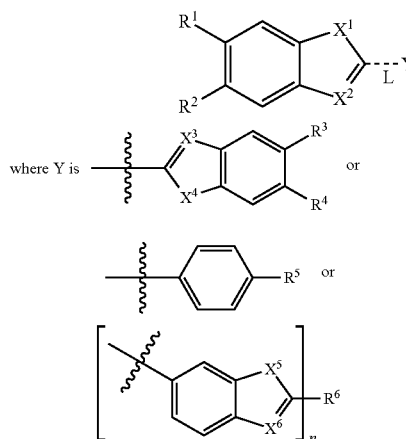
[0175] To the extent necessary to understand or complete the disclosure of the present invention, all publications, patents, and patent applications mentioned herein are expressly incorporated by reference therein to the same extent as though each were individually so incorporated.

[0176] Having thus described exemplary embodiments of the present invention, it should be noted by those skilled in the

art that the within disclosures are exemplary only and that various other alternatives, adaptations, and modifications may be made within the scope of the present invention. Accordingly, the present invention is not limited to the specific embodiments as illustrated herein, but is only limited by the following claims.

We claim:

1. A method of inhibiting the activity of Botulinum neurotoxin A metalloprotease which comprises contacting Botulinum neurotoxin A metalloprotease with at least one compound having the following structural formula:

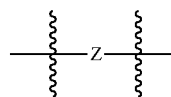


wherein

n is 1 or 2;

X¹, X², X³, X⁴, X⁵ and X⁶ are each independently N, S, O, SO₂, CR⁷ or NR⁸ and at least one of X¹ or X² is N, S, O, SO₂, or NR⁸;

L is a linker which may be a direct bond or



where Z is an optionally substituted alkyl, alkenyl, dialkenyl, trialkenyl, or aryl, or C(O)NH; and

R¹, R², R³, R⁴, R⁵, R⁶ and R⁷ are each independently hydrogen, amino, amine with stabilized carbocations, carboxyl, optionally substituted alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, aryloxy, cycloalkoxy, heteroaryloxy, alkoxy-carbonyl, alkylamino, carbamoyl, alkylaminocarbonyl, alkylsulfhydryl, alkylhydroxymate; and

R⁸ is hydrogen, OH, a halogen, or an optionally substituted alkyl.

2. The method of claim 1, wherein at least one of R¹, R², R³, or R⁴ is hydrogen, amidine, 2-imidazoline, amino, guanidine, methyl, aminomethyl-hydroxamine, or methylamine-guanidine.

3. The method of claim 1, wherein R⁵ is hydrogen, amidine, 2-imidazoline, amino, guanidine, methyl, aminomethyl-hydroxamine, methylamine-guanidine, 4-oxy-benzamidine, 1H-indole-6-carboxamide, or 1H-indole-5-carboxamide.